Autoxidation of Fatty Materials in Emulsions. I. Pro-oxidant Effect of Histidine and Trace Metals on the Oxidation of Linoleate Esters[1]

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Abstract

Aqueous emulsions of methyl or ethyl linoleate (sodium dodecylsulfate as emulsifier) together with such added components as l-histidine, metal chlorides, buffers, and acid or alkali, were oxidized in the dark with shaking in an oxygen atmosphere. Under optimum conditions (pH 6.5), the linoleate peroxide content, after 2 hr autoxidation at 20C, was increased more than 3-fold by the addition of 1 ppm of ferrous or ferric ions, approximately 20-fold by a 0.01 M concentration of histidine and more than 60-fold by the addition of both histidine and ionic iron. The pro-oxidative effect of other transition metal ions (Cu^{++}, Co^{++}, Cr^{+++}, Mn^{++}, and Ni^{++}) also was investigated. None of these ions had a significant effect alone. Combined with 0.01 M histidine, only Mn^{++} increased peroxidation over that when when histidine alone was added.

The pro-oxidative action of histidine was retarded approximately 60% by 0.1 N acetate buffer and completely repressed by 0.05 M phosphate, nonionic emulsifiers, and low and high pH. The threshold concentration of histidine necessary for pro-oxidative action was greater than 0.0001 M.

The pro-oxidative activity of histidine in linoleate emulsions is thought to be due to the formation of pro-oxidant complexes with trace quantities of ionic iron. The solvation of transition metal ions with water or chelation with histidine either enhances or reduces their pro-oxidative efficiency depending on the electronic configuration of the metal ion.

Introduction

AUTOXIDATION of fatty materials is a complicated phenomenon (1) even when it involves only the oxidation of an anhydrous polyunsaturated fatty acid ester under carefully controlled conditions. Autoxidation of fats in food is further complicated because fat is present as a diverse mixture of many lipids. The fat is often in finely divided or emulsified state, intimately associated with a mixture of solid components and a complex aqueous phase containing a variety of both soluble and colloidally dispersed materials. In such a system, numerous reactions, both oxidative and otherwise, occur simultaneously, and the rate, extent, and possibly the course of reactions with oxygen are influenced by numerous compounds (8, 9,10) which promote or inhibit oxidation. Considerable work has been published on the influence of amino acids on the autoxidation of fats.

Nearly all of the known amino acids have been reported to have pro-oxidative and/or antioxidative activity. Cysteine (2,7,13,22) was found to be strongly pro-oxidative over a wide range of conditions but antioxidative (13) at pH 9.5. Franke (2) reported a number of amino acids to be strong pro-oxidants in linoleic acid emulsion. The most effective, in decreasing order, were: histidine, arginine, cysteine, lysine, and tryptophane. He also found proline to be a highly effective pro-oxidant in anhydrous linoleic acid while Janicki et al. (7) found valine to be second only to cysteine in promoting the autoxidation of anhydrous lard. Marcuse (14) found some of these same amino acids to be effective antioxidants in emulsion. The most effective, in decreasing order, were: histidine, tryptophane, threonine, lysine, arginine, phenylalanine, and serine. Kaufmann (10) has found dihydroxyphenylalanine to be an exceptionally effective antioxidant in potassium linoleate emulsions. Janicki et al. (7) reported the following amino acids, in decreasing order of effectiveness, to be strong antioxidant synergists for α-tocopherol in anhydrous lard: serine, isoleucine, alanine, lysine, histidine, and tyrosine.

In the present paper, evidence is presented showing that whether histidine acts as a pro-oxidant or antioxidant depends on pH, concentration, and the presence or absence of metal ions, phosphate and nonionic emulsifiers. The importance of the interaction of trace metal ions and histidine is emphasized.

Experimental

Materials. Methyl linoleate was prepared by the methanolysis of safflower oil, and purified by a modification of the method of Parker et al. (17) using a ratio of 1:1.6:4, respectively, of safflower oil methyl esters, urea, and methanol. The final product was fractionally distilled through a 75 cm Vigreaux column under 0.8 mm pressure. It was 97.3% linoleate with I.V. 172.8, peroxide value 2.6, and 0.5% conjugated diene. Ethyl linoleate employed in some early experiments was prepared by a similar procedure. It was 99.6% linoleate ester with I.V. 164.1, peroxide value 1.7, and 0.05% conjugated diene.

1-Histidine was a high purity grade obtained from the Nutritional Biochemicals Corporation. All buffers, salts, acids, and bases were reagent grade chemicals. Metals were used as their chloride salts. Sodium dodecylsulfate (emulsifier) was a highly purified laboratory preparation.[3] Other emulsifiers, used in exploratory experiments, included purified laboratory preparations[3] of sodium myristate, potassium palmitate, and ethenoxylated tetradecanol (averaging 15 ethenoxy groups per molecule) and two commercial

[1] Presented in part at the AOCS meeting, St. Louis, Mo., 1961.

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[3] We are indebted to members of the Detergents Investigations in this laboratory for these emulsifiers.

emulsifiers, Tween 20 and Span 20. Redistilled water was used in preparing all emulsion components. It was prepared by redistilling a solution of potassium hydroxide and potassium permanganate in laboratory distilled water in an all-glass still.

Apparatus. Oxidation studies were made in two types of apparatus. Oxygen absorption investigations were made in Warburg apparatus, using the same 90 ml reaction flasks and manometers as described previously (20). In a larger proportion of our experiments, chemical changes in the autoxidation of emulsified linoleate esters were studied using 270 ml glass vessels that we have designated as rocker tubes. These vessels consisted of cylinders with rounded ends and an open-end side-arm attached at right angles. The side-arms were sealed during autoxidation by clamping short pieces of rubber tubing, previously cleaned by boiling first in sodium hydroxide solution and finally in distilled water.

Procedure. All experimental work, wherever feasible, was carried out in all-glass equipment to minimize metal contamination. However, in the preparation of the initial concentrated emulsions, a mixture consisting of 4 ml of methyl linoleate, 25 ml of 0.5% sodium dodecylsulfate solution, and 21 ml of water was emulsified for 15 min in a Virtis 45 homogenizer in contact with teflon and stainless steel components of the homogenizer assembly. Ten ml of this emulsion was transferred to each of 4 rocker tubes and diluted to 25 ml with water and/or test solutions. Stable emulsions of comparatively uniform oil particles, averaging less than 1μ in size were obtained. A typical unbuffered control contained 0.0024 mole of methyl linoleate, 0.1% sodium dodecylsulfate, and 24.2 ml of water, and had a pH of 6.5. Test components included buffers, standard solutions of hydrochloric acid and sodium hydroxide, metal chlorides, and l-histidine. Solutions of these components were added as required in amounts predetermined to give the appropriate pH and concentration in 25 ml of emulsion. Solutions of the pro-oxidant test components (metal salts and histidine) were added last.

As soon as the test mixtures were prepared, the gas in each rocker tube was evacuated and replaced with pure oxygen to a final pressure of 1 atm plus 100 mm Hg and the tube sealed. The rocker tubes were then mounted in a parallel, horizontal position, with the side arm upright, in an Equipoise shaker in a constant temp room at 20C. The tubes were shaken at 160 strokes per min for 2 hr in the dark. Each emulsion was then extracted once with 75 ml of 2:1 ethyl ether-ethanol and the extract washed with 100 ml of distilled water. The autoxidized methyl linoleate was recovered by evaporation in a rotary evaporator under reduced pressure. Tests have shown that this method of recovering the autoxidized methyl linoleate resulted in no significant change in the peroxide content of the sample. Approximately 0.05 g was used for the determination of conjugated diene by ultraviolet spectroscopy and the remainder used for peroxide determination (19). The reduction product from the peroxide determination was recovered and used for determining iodine number by the Wijs method.

In our present rapid autoxidations, we found it expedient to use methyl linoleate having a small peroxide content. Samples of 8 to 9 g, having an initial peroxide value of 2.6, were stored under nitrogen in sealed ampoules at −12C. The samples when opened had an average peroxide value of approximately 12.

TABLE I

The Effect of Histidine, Buffer, and pH on the Autoxidation [a] of Methyl Linoleate

Histidine molarity	pH	Buffered emulsions		Unbuffered emulsions		
		Buffer 0.1 N	Peroxide value	Acid or alkali	Normality × 10^2	Peroxide value [d]
0	4.3	Acetate [b]	41	HCl	0.01	40
0.01	4.3	Acetate	55	HCl	0.92	56
0	6.5	Acetate	70	O	0	26
0.01	6.5	Acetate	198	HCl	0.24	521
0.01	7.3	....	....	O	0	416
0	8.8	Tris [c]	19	NaOH	0.0032	27
0.01	8.8	Tris	91	NaOH	0.32	100

[a] Two hr autoxidation in rocker tubes at 20C in the dark.
[b] Acetic acid-sodium acetate buffer.
[c] Tris(hydroxymethyl)aminomethane-hydrochloric acid buffer.
[d] Milliequivalents of peroxide oxygen/kg of sample.

To minimize the effect of slight differences in peroxide content of linoleate samples and unknown variations between experiments, each series of experiments involving the effect of histidine and other variables, such as pH and traces of metal ions, was carried out in randomly designed experiments (23) and replicated in additional random series. We were able thus to repeat the experiments with reasonable consistency. The data in Tables I and II represent the average of two or more replications.

In oxygen absorption studies in the Warburg apparatus, 5 ml of a similar concentrated emulsion was transferred to 90 ml reaction flasks and diluted to 10 ml with water and/or solutions of test components. The final concentration of all components was the same as those employed in the rocker tubes. After the emulsions were prepared, the reaction vessel-manometer assemblies were flushed with oxygen for 5 min, and then transferred to the Warburg bath held at 30C. The flasks were equilibrated 10 min and then sealed. Autoxidation was carried out in the dark. Mercury was used as the manometer fluid and the flasks of the thermobarometers contained 10 ml of water.

Results

Effect of Buffers and pH. In early studies of the pro-oxidative action of histidine, we conducted a wide variety of exploratory experiments using emulsified ethyl linoleate as substrate and a 22 hr oxidation period at 20C in the dark. The catalytic effect of histidine was extraordinary, resulting in peroxide values above 2700. Four other amino acids (l-proline, l-hydroxyproline, d,l-tryptophane and d,l-methionine) either had no effect or, at most, no more than 10% of that of histidine.

The presence of 0.01 M histidine, in otherwise unbuffered emulsion, resulted in an initial pH of 7.3. Due to the considerable buffering action of the free amino acid, the emulsions were resistant to changes in hydrogen ion concentration. Nevertheless, due to widespread oxidation at peroxide values of 2700 and increasing concentrations of acidic fragments, the

TABLE II

The Effect of Histidine and Cupric, Ferric, and Ferrous Ions on the Autoxidation [a] of Methyl Linoleate

Histidine molarity	Trace metals, ppm	Peroxide values [b]					
		Cupric		Ferric		Ferrous	
		pH 6.5	4.3	6.5	4.3	6.5	4.3
0	0	26	40	28	41	25	39
0.01	0	483	56	528	62	536	49
0	0.1	30	40	33	61	27	69
0	1	34	41	115	114	88	124
0.01	0.1	476	58	826	67	1150	79
0.01	1	507	60	1731	125	1870	172

[a] Two hr autoxidation in rocker tubes in the dark at 20C.
[b] Milliequivalents peroxide oxygen/kg of sample.

pH of an emulsion decreased by nearly one unit. In the present less extensive 2 hr oxidation experiments, the change in pH was 0.2 unit or less. The controls, although not protected by the buffering action of histidine, undergo little autoxidation and consequently little change in pH.

In order to study what effects changes in pH might have on autoxidation of linoleate in emulsion, the effect of buffers was investigated. Phosphate buffers, commonly used in emulsion studies to control pH over a wide range, were found to suppress the catalytic action of histidine. The effect of pH was then studied in two series of experiments. In a 0.1 N buffer series, acetic acid-sodium acetate was used to control pH in ranges below 7 and tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris) above 7. The buffer was omitted in another series of comparable emulsions and acid or alkali was used to adjust the hydrogen ion concentration. The max pro-oxidative action of histidine was obtained in both series of emulsions at pH 6.5 (Table I). In the unbuffered emulsions, however, the increase in autoxidation at this pH was much greater than in buffered emulsions, the presence of 0.1 N acetate having retarded the pro-oxidative action of histidine by approximately 60%. Additional autoxidations of emulsified linoleate buffered with 0.1 N acetate at pH 7.3, 7.1, 6.5, and 5.5 indicated that this buffer retarded the oxidation proportionately over the entire range and that the optimum catalysis by histidine was obtained at pH close to 6.5. Acetate failed to retard autoxidation only at low pH (4.3) where histidine had little pro-oxidative effect.

Catalysis by Histidine and Trace Metals. The catalytic action of histidine could possibly be due to its interaction with other components of the emulsion, such as trace metals which are extremely difficult to completely eliminate. Linoleic acid (24) distilled in all-glass apparatus and purified methyl linoleate (25) have been reported to contain traces of copper, iron, and cobalt. It is also difficult to obtain amino acids containing less than 1–3 ppm of various trace metals particularly if the amino acid is prepared from biological sources.

In view of the known ability of histidine (3) to complex with metal ions and oxygen, rocker tube experiments were conducted with 0.1 M histidine and 0.1 and 1 ppm of cupric (Cu^{++}), ferric (Fe^{+++}), and ferrous (Fe^{++}) ions at pH 4.3 and 6.5. The results are shown in Table II. The pH of the methyl linoleate emulsions was adjusted with the same amounts of hydrochloric acid as used in the previous series. Oxygen absorption experiments in the Warburg apparatus with comparable unbuffered emulsions using 1 ppm of the above metal ions are shown graphically in Figure 1. The pH of emulsions without histidine was 6.5, and with histidine, 7.3.

In the 2 hr rocker tube experiments (Table II), the cupric ion (Cu^{++}) had no appreciable effect on the increase in linoleate peroxide content either in the absence or presence of histidine. Ferrous (Fe^{++}) and ferric ions (Fe^{+++}), on the other hand, had a significant effect (based on statistical analysis) in concentrations of 1 ppm at both pH 4.3 and 6.5. In combination with histidine at pH 6.5, the increase in catalytic efficiency was especially striking. It is interesting to note that the linoleate peroxide content was increased more than 3-fold by the addition of 1 ppm of Fe^{++} and Fe^{+++}, approximately 20-fold by a 0.01 M concentration of histidine and more than 60-fold by the addition of both histidine and ionic iron. At pH 4.3, however, there was little pro-oxidative interaction of this amino acid with iron.

Similar results were obtained in the Warburg oxygen absorption experiments (Figure 1). Methyl linoleate in control emulsion oxidized slowly, adding only 0.071 moles of oxygen per mole of ester in 48 hr. The addition of 1 ppm of Cu^{++} had little effect in the first few hr but on prolonged oxidation to 48 hr, there was a definite pro-oxidative effect with the absorption of 0.25 moles of oxygen. The effect of adding Fe^{++} or Fe^{+++} was more than double that of copper. Emulsions containing histidine absorbed a mole of oxygen per mole of linoleate in 35 hr. Adding Cu^{++} shortened this period to 32 hr; Fe^{+++}, to 10.5 hr; and Fe^{++}, to 6.5 hr. Ferrous and ferric ions would be expected to have a similar catalytic effect due to the rapid oxidation of ferrous to ferric (27). However, combined with histidine, the ferrous ion had a greater catalytic effect than the ferric ion. The effect of either ion combined with histidine was immediate and rapid.

The oxygen absorption curves in Figure 1 represent an average of 8 determinations each for the control emulsions and those with added Fe^{++} or Fe^{+++}, 4 with added Cu^{++}, and only 2 each for the emulsions containing histidine with or without added metal ions. In the absence of histidine, it was necessary to make use of a larger number of determinations to obtain reliable data.

A limited number of rocker tube experiments, similar to those reported in Table II, were carried out employing other metal ions from the same transition series. Two hr oxidations at 20C and pH 6.5, of methyl linoleate emulsions containing 1 ppm of chromic, cobaltous, manganous, or nickelous ions, did not differ significantly from that of the controls. In comparable oxidations, however, in which 0.01 M histidine was present, the pro-oxidative action of this amino acid was increased 20% by the manganous ion and inhibited approximately 50% and 20%, respectively, by cobaltous and chromic ions. The nickelous ion had no effect.

The Effect of Emulsifiers. In early rocker tube studies with ethyl linoleate, it was found that a non-ionic emulsifier, ethenoxylated tetradecanol, repressed the pro-oxidative action of histidine. Further investigation using oxygen absorption studies confirmed this and showed that Span 20 and Tween 20 had a similar effect. It was further noted that ionic emulsifiers such as potassium myristate, sodium pal-

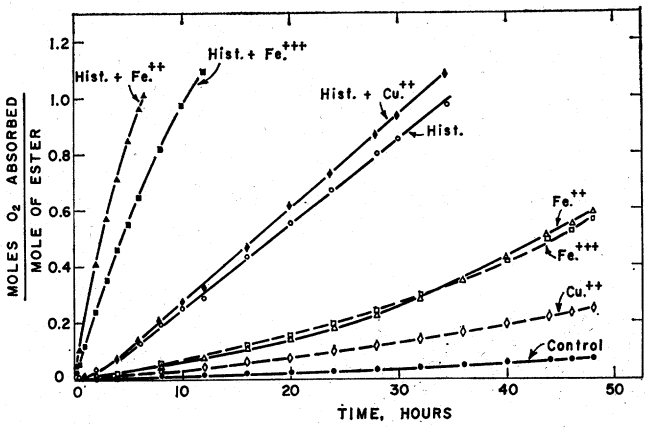

FIG. 1. The effect of histidine (Hist), cupric (Cu^{++}), ferric (Fe^{+++}), and ferrous (Fe^{++}) ions on the absorption of oxygen by emulsified linoleate in the dark at 30C.

mitate and sodium dodecylsulfate supported the pro-oxidative action of histidine. This will be discussed in greater detail in a subsequent paper.

Other Analyses. As stated previously, analytical values for conjugated diene and iodine number were obtained in the rocker tube experiments. These indicated parallel changes in the autoxidized linoleate molecule in agreement with peroxide values. Conjugated diene content increased less rapidly indicating possibly the formation of some diperoxide. I.V. decreased proportionately reflecting a disappearance of double bonds due to secondary oxidation and decomposition of hydroperoxide.

Discussion

Interaction of Histidine and Trace Metal Ions. The powerful interaction of histidine with added traces of ionic iron strongly suggests the possibility of an iron-histidine complex as the primary pro-oxidant in catalyzing the autoxidation of emulsified linoleate. The activity of histidine, when no iron is added, could then be accounted for as a complex formed with iron present as a trace impurity. It would seem safe to assume that contamination of the emulsion with as little as a few 10ths ppm of free ferrous or ferric ion would be sufficient to form a chelate with histidine and account for its activity as an autoxidation catalyst.

The order of relative pro-oxidative efficiency of metals in the first transition series is often reversed, depending, at least in part, on whether they exist potentially as free ions or as complexes with chelating amines or with polar solvents. This particularly applies to cobalt and iron. Zettlemoyer and Myers (28) found cobaltous ions much superior to those of iron in the autoxidative drying of linseed oil. Chelation with amines such as o-phenanthroline reduced the effectiveness of cobalt but strongly enhanced that of iron.

In the autoxidation of linoleate in benzene solution, Uri (24,25) found cobaltous stearate was a highly effective catalyst but ferrous and ferric stearates were not. With the addition of a high proportion of polar nitrobenzene, however, cobaltous stearate was no longer effective. Iron was more effective than cobalt when complexed with an azaporphin such as phthalocyanine (4,24,25). The powerful catalysis of the autoxidation of lipids by analogous, naturally occurring iron porphyrin complexes such as heme also has been studied intensively (21).

According to Myers and Zettlemoyer (16) the uncomplexed cobaltous ion with 3 unpaired electrons in the d-shell is able to form an activated reversible complex with oxygen involving min changes in energy. In this condition, it is a highly effective catalyst. However, when the cobaltous ion is 1) solvated with either polar nitrobenzene (24,25) or water molecules as in our studies, or 2) is chelated with histidine or o-phenanthroline (16,28), it no longer has as favorable an electron configuration due to sharing of electrons which fill the d-shell or involve other electron orbitals. On the other hand, ionic iron, when solvated with water or chelated with histidine, azaporphins (24,25), naturally occurring porphyrins (21) or o-phenanthroline (16,28), shares electrons with these ligands which provide it with a d-shell configuration approaching that of the catalytically effective free cobalt ion. The contribution of the molecular structure of the ligand to catalytic efficiency, however, is still imperfectly understood.

The observed pro-oxidative action in pure linoleate emulsions with only histidine present is thought to be due to an iron-histidine complex, iron being present as a trace impurity. The solvation of transition metal ions with water or chelation with histidine either enhances or reduces their pro-oxidative effect, depending on the electronic configuration of the metal ion. In general, the environment of the metal ion in the oxidation system, such as solvent, amino acid, emulsifier, buffer, etc., is important in determining pro-oxidative efficiency.

Effect of pH and Histidine Concentration. Our investigations have shown that histidine alone or combined with iron, has a much reduced pro-oxidative action at 0.001 M concentration and no appreciable effect at 0.0001 M. Marcuse (15) has found that histidine, at low concentrations, behaved as an antioxidant in the autoxidation of emulsified linoleate and a pro-oxidant at higher concentration. Studies by Hearon et al. (3) on the chelating of ferrous (Fe^{++}) and ferric ions (Fe^{+++}) with histidine provides an explanation for these observations. The Fe^{++}-histidine chelate was found to be unstable, and histidine in low concentrations was not able to compete successfully with the hydroxyl ion (OH^-). In the presence of oxygen, the chelated Fe^{++} was rapidly oxidized to Fe^{+++}. The competition of OH^- for Fe^{+++} proved to be so strong that they were unable to demonstrate the formation of a ferric chelate at low ratios of histidine to Fe^{+++} (approximately 4:1). In experiments for which data are given in our paper, the ratio was never less than approximately 500:1. With so high a ratio, the pro-oxidative iron-histidine chelate would be expected. In other experiments when the concentration was reduced from 0.01 M to 0.0001 M, the resulting ratio of 5:1 closely approximated that studied by Hearon et al. (3). At 0.0001 M concentration, no chelate would be formed and no pro-oxidative action was found. When histidine is incapable of complexing with iron, it appears to be a true antioxidant. This is suggested by the fact that a number of conditions under which Marcuse (15) has found it to behave as an antioxidant, are those where the chelate would not be expected to exist, namely, low histidine concentrations and high pH.

Concentration also appears to explain the effect of pH. With increasing pH, the concentration of OH^- also increases, and thus reduces the effective concentration of Fe^{++} or Fe^{+++}. At lower pH (<5.2) Hearon et al. (3) have shown that histidine as its hydrochloride was unavailable for chelate formation. These facts are consistent with the picture that optimum catalysis takes place where the product of the concentrations of available free metal ion and free histidine base is maximum, namely at pH 6.5.

The Effect of Phosphate and Emulsifiers. Early in our investigations, phosphate buffer

$$(NaH_2PO_4–Na_2HPO_4)$$

was found to repress the pro-oxidative action of histidine. Recently we have confirmed the findings of Marcuse (15) that histidine in the presence of phosphate is an antioxidant. Although the inhibition of autoxidation by phosphate was reported by Hopkins (5) as early as 1925, the nature of its action is still debatable. Phosphate (26) is known to bind ions of transition metals firmly. Thus, the simplest explanation of its effect in the present experiments is that it complexed metal contaminants, leaving histidine free to act as an antioxidant. Other workers (18), how-

ever, have reported that phosphoric acid inhibited autoxidation by complexing hydroperoxides.

The effect of emulsifiers on the pro-oxidative action of histidine is not understood. Non-ionic emulsifiers appear to repress this action owing possibly either to their ability to complex metal contaminants or to react with free radicals, thus acting to terminate the autoxidative chain reaction. Ionic emulsifiers, such as sodium dodecylsulfate may promote the autoxidation by combining with the metal ions and concentrating them at the linoleate-water interface.

Effect of Acetate Buffers. No adequate explanation is available to explain the retarding action of 0.1 N acetate. Further experiments have shown that the effect of histidine on the autoxidation of emulsified methyl linoleate is inhibited approximately 50% by 0.025 N acetate or 0.05 N sodium chloride. Mabrouk et al. (11) also have reported that the oxidation of emulsified methyl linoleate is inhibited by sodium chloride with maximum inhibition at concentrations of 5.85 moles of sodium chloride/mole methyl linoleate (approximately 0.6 N by calculation from their data). They attributed the effect of sodium chloride to decreased solubility of oxygen in the emulsion. However, considering the low concentration of the two salts in our experiments and the magnitude of the inhibition, decreased solubility of oxygen would not appear to be involved.

Mechanism of Metal Catalysis. The autoxidation of unsaturated fatty materials involves a series of free radical reactions, comprising 3 steps: initiation, propagation, and termination. Although the importance of trace metals in catalyzing the initiation step directly has been open to question, Uri (4,24,25) recently has cited considerable evidence to support his hypothesis that autoxidation in the initial stages is a trace-metal-catalyzed reaction. Ingold (6) also has reviewed the evidence based on the use of other types of organic substrates.

All investigators are in agreement that once a small amount of hydroperoxide has been formed, the catalytic efficiency of a trace metal is dependent largely on its ability to catalyze the decomposition of hydroperoxide. According to Walling (27), the decomposition of a hydroperoxide (ROOH) by metal ions in their lower (M^{n+}) and higher state ($M^{(n+1)+}$) of oxidation involves two processes:

$$(1)\quad M^{n+} + ROOH \rightarrow M^{(n+1)+} + RO\cdot + OH^-$$
$$(2)\quad M^{(n+1)+} + ROOH \rightarrow ROO\cdot + H^+ + M^{n+}$$

The free radicals RO· and ROO· then initiate new chain reactions involving the unoxidized substrate. It was noted in our investigations that the level of the initial peroxide content influences the pro-oxidative action of histidine. This would indicate that histidine, probably complexed with ionic iron, played an important part in hydroperoxide decomposition. Whether it also plays a part in the first initiation step must await further studies with a peroxide-free substrate.

Metal ions of the first transition series may also act to terminate the free radical chain by forming complexes with one or more peroxy radicals, ROO·, giving rise to molecular decomposition products. Ingold (6) has reviewed this subject extensively citing investigations both in the autoxidation of fats and of other types of organic substrates. Chain termination is associated primarily with the lower oxidation state of a metal ion. This may explain the decreased pro-oxidative effect when histidine and 1 ppm of cobaltous ion (Co^{++}) were both present in the linoleate emulsion. Cobalt (present as the free ion or as a histidine complex) may form chain-terminating, non-radical decomposition products when it reacts with peroxy radicals and thus appears to inhibit the pro-oxidative action of histidine. By the same reasoning, transition metal ions which in our present investigation appear to have had little pro-oxidative interaction with histidine, may as chelates actually exist in a dynamic balance acting both as pro-oxidants and free radical chain-terminators. The shift in this balance would then depend on the electronic configuration of the d-shell and the nature of the chelating ligand.

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